Tetrahedron Letters,Vol.24,No.19,pp 2011-2014,1983 0040-4039/83/192011-04$03.00/0
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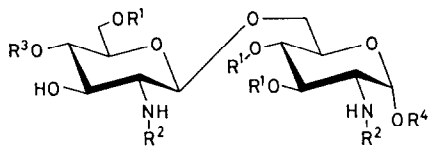
SYNTHETIC APPROACH TO LIPID A: PREPARATION OF PHOSPHORYLATED DISACCHARIDES CONTAINING (R)-3-HYDROXYACYL AND (R)-3-ACYLOXYACYL GROUPS

M. Inage, H. Chaki, M. Imoto, T. Shimamoto
S. Kusumoto, and T. Shiba*
Department of Chemistry, Faculty of Science, Osaka University
Toyonaka, Osaka 560, Japan

Summary: A synthetic route was established to construct the proposed structure of lipid A which consists of β(1-6) glucosamine disaccharide 1,4'-diphosphate O,N-acylated with (R)-3-hydroxy- or (R)-3-acyloxytetradecanoic acid.

Lipid A is a lipophilic part of the cell surface lipopolysaccharide (LPS, endotoxin) of Gram-negative bacteria and is known to be responsible for most of the biological activities exerted by LPS. The basic structure of lipid A from *Salmonella* and some other species was proposed as 1, which consists of O,N-polyacylated β(1-6)glucosamine disaccharide 1-α,4'-diphosphate. Among the acyl groups, (R)-3-hydroxytetradecanoyl group is the typical and predominant one. It bounds to the two amino and also to some of the hydroxyl groups. Considerable amounts of normal long chain acyl groups are also detected. They may be present in (R)-3-acyloxyacyl forms. However, neither the details on the positional distribution of acyl groups nor the relationships between chemical structures and biological activities are known yet.[1)]



Compd	R^1	R^2	R^3	R^4	mp(°C)	$[\alpha]_D$
1	H, acyl		P	P	natural lipid A	
2	C_{14}	C_{14}	P	P	previous work[2)]	
3a	C_{14}	$C_{14}OH$	H	H	192-195*[3]	+ 0.41°*[4]
3b	$C_{14}OH$	$C_{14}OH$	H	H	203-204	- 4.7° *[4]
3c	$C_{14}OH$	$C_{14}OC_{14}$	H	H	176-179	+ 6.7° *[4]
4b*[1]	$C_{14}OH$	$C_{14}OH$	P	H	187-189*[3]	- 7.2° *[5]
4c*[2]	$C_{14}OH$	$C_{14}OC_{14}$	P	H	183-189	+ 7.5° *[5]
5a*[1]	C_{14}	$C_{14}OH$	H	P	177-181	+13.5° *[5]
6a*[1]	C_{14}	$C_{14}OH$	P	P	218-222*[3]	+12.5° *[6]

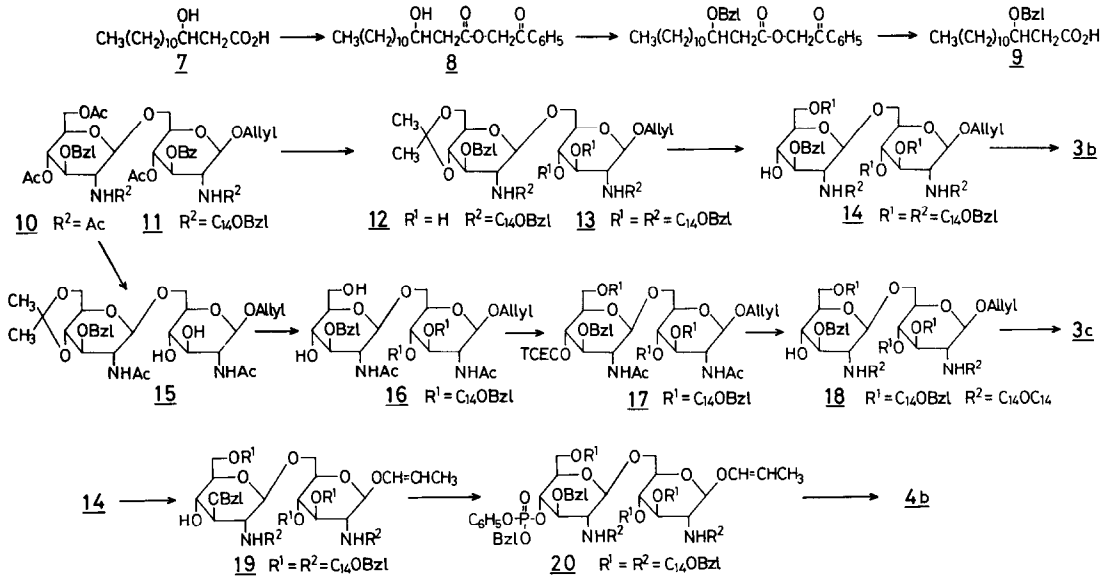
C_{14} : tetradecanoyl, $C_{14}OH$: (R)-3-hydroxytetradecanoyl
$C_{14}OC_{14}$: (R)-3-tetradecanoyloxytetradecanoyl, P : $PO(OH)_2$

*[1] Triethylamine salt. *[2] Na salt. *[3] With decomposition. $[\alpha]_D$'s were measured at *[4] c 0.3-1.0 in $CHCl_3$-MeOH (5:1), *[5] (3:1), or *[6] $CHCl_3$-MeOH-H_2O (30:10:1).

We recently reported a synthesis of 2 which contained all proposed structural elements except that the acyl groups are simple tetradecanoyl.[2] However, 2 and related compounds showed so far only few of lipid A activities. This might indicate the importance of the 3-hydroxyacyl or 3-acyloxyacyl moieties for the activities. Therefore, we next attempted to synthesize the lipid A structure containing these acyl groups and phosphate groups. The target structures in this study were O-acyl-N-hydroxyacyl, O,N-hydroxyacyl, and O-hydroxyacyl-N-acyloxyacyl disaccharides (3a-c) and their phosphates.

First of all, the hydroxyl group in optically pure (R)-3-hydroxytetradecanoic acid (7)[3] was protected by benzylation as follows. The acid 7 was converted into the phenacyl ester 8 (98%, mp 68-71°C, with phenacyl bromide - Et_3N in ethyl acetate). On reaction of 8 with BzlBr - Ag_2O in benzene[4] (at room temperature for 48h), 3-O-benzyl derivative was obtained, which was treated with Zn-powder in acetic acid (at 50°C for 3h) to give the desired (R)-3-benzyloxy acid 9 (syrup, 78% from 8). A similar yield of 9 could be also obtained with benzyl trichloroacetimidate-CF_3SO_3H[5] in CH_2Cl_2 within much shorter reaction time.

By using this protected acid 9, two of the above target compounds, 3a and 3b, could be prepared from the same disaccharide 10[2] in a manner similar to that described for 2.[2] After removal of the both N-acetyl groups of 10 [i) $Et_3O\cdot BF_4$ in CH_2Cl_2 in the presence of anhydrous K_2CO_3 at room temperature, ii) 1N HCl in THF], the resulting free amino groups were acylated with 9 and dicyclohexylcarbodiimide(DCC) (in THF-$CHCl_3$ 5:1) to afford 11 (49% from 10, mp 190-192°C, $[\alpha]_D^{22}$ -4.76°). It was then converted into an isopropylidene derivative 12 [i) conc. NH_4OH-EtOH (1:3) at 50°C 36 h, ii) 2,2-dimethoxypropane - TsOH in DMF; 62% from 11, mp 172-175°C], whose two hydroxyl groups were

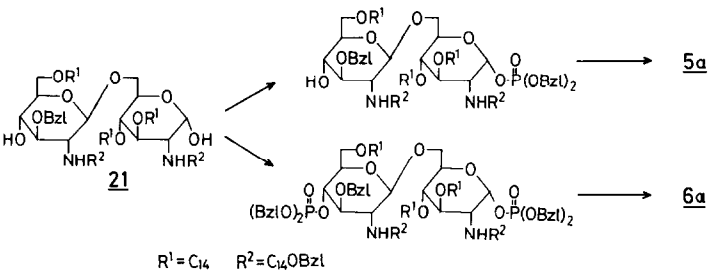


acylated [with 9 and DCC-dimethylaminopyridine(DMAP) in CH_2Cl_2 at room temperature for 12h] to give 13 (73%, mp 93-95°C). The isopropylidene group was removed (90% acetic acid at 90°C for 20 min) and the primary hydroxyl group was acylated with the same reagents as above (at 0°C for 5h). The product 14 (69% from 13, mp 59-64°C) was deprotected [i) cleavage of the allyl group in a usual way,[2,6,7] ii) hydrogenolysis with Pd-black in THF-ethanol] to give 3b (68% from 14). Use of tetradecanoyl chloride in place of 9 and DCC in the above synthesis afforded 3a, which was identical with that obtained previously by an alternative way.[8]

By contrast, a slight modification of the synthetic route was necessary for the preparation of an N-(3-acyloxyacyl)-type disaccharide 3c. Since the acyloxyacyl group already contains an ester function in it, ester-type O-protecting groups cannot be removed selectively. Therefore, the synthesis of 3c was performed, as shown in the scheme, by keeping the both N-acetyl groups of the starting disaccharide 10 until all the long-chain O-acyl groups had been introduced to the desired position and the 4'-hydroxyl function protected with the 2,2,2-trichloroethoxycarbonyl (TCEC) group.[9] Thus, after the all O-acyl protecting groups of 10 had been removed (conc. NH_4OH-MeOH 1:2, at 50°C for 1 h), the product was converted into the isopropylidene derivative 15 (53% from 10, mp 208-212°C dec). 3,4-O-Acylation (9 and DCC-DMAP in CH_2Cl_2) and hydrolysis of the isopropylidene group as above afforded 16 (41%, mp 171-173°C). 6'-O-Monoacylation followed by trichloroethoxycarbonylation of the 4'-hydroxyl group (TCEC chloride in pyridine at room temperature for 3 h) afforded 17 as a homogeneous syrup after purification with a silica gel column. Both N-acetyl groups were then removed (Meerwein's reagent and then aqueous HCl as described above), and the resultant free amino groups acylated with (R)-3-tetradecanoyloxytetradecanoic acid and DCC (in THF-$CHCl_3$ 4:1 at room temperature for 2 days). Cleavage of TCEC group (with Zn-powder in AcOH at room temperature for 2 h) gave 18 (48% from 16, mp 111-113°C). Removal of the allyl group in a usual manner yielded the O-hydroxyacyl-N-acyloxyacyl-type disaccharide 3c (68%).

4'-Phosphates of the acyl disaccharides could be prepared in the same way as described for the corresponding O,N-tetradecanoyl derivative.[2] For example, after isomerization of the allyl group of 14, the 1-propenyl glycoside 19 was treated with phenyl dihydrogen phosphate and DCC (in dry pyridine at room temperature for 24h). The crude reaction product was treated with phenyl diazomethane and subjected to silica gel column chromatography to give benzyl phenyl ester of 4'-phosphate 20.[10] Deprotection of 20 [i) HgO-$HgCl_2$ in aqueous acetone, ii) hydrogenation first with Pd-black then with PtO_2 in THF-ethanol] gave desired 4'-monophosphate (4b) (36% from 14). The 4'-monophosphate 4c of N-acyloxyacyl-type disaccharide could be also prepared similarly from 18.

Preparation of 1-α-phosphates of N-(3-hydroxyacyl) or N-(3-acyloxyacyl)-glucosamine derivatives had failed with the conventional methods such as the oxazoline procedure. However, this problem could be solved by use of our new



efficient phosphorylation procedure with BuLi and a phosphorochloridate.[11] Compound 21 (mp 162°C with sintering at 145°C), which was obtained as a synthetic intermediate to 3a,[12] was first treated with BuLi (1 equivalent in dry THF at -70°C) and then with dibenzyl phosphorochloridate. After the mixture had been stirred for 5 min at -70°C and then for 5 min at -50°C, acetic acid was added, and the whole mixture was immediately subjected to hydrogenolysis (with Pd-black) to afford 1-α-monophosphate 5a (65% from 21) after purification with a silica gel column ($CHCl_3$-MeOH-H_2O 40:10:1).

In the above synthesis of 5a, the more acidic hydroxyl group on C-1 could be quite selectively phosphorylated via lithium salt with 1 mole of BuLi, though another free hydroxyl group was present on C-4'. However, both hydroxyl groups of 21 could be also phosphorylated simultaneously by using each 2 moles of BuLi and the chloridate under an otherwise similar condition.[11] The corresponding 1-α,4'-diphosphate 6a was obtained (23% from 21) in this way.

The structures and physical properties of lipid A analogs so far prepared are summarized in the table. As seen from the table, it became now possible to prepare acyl disaccharide diphosphates corresponding to the proposed structure of lipid A with any combination of acyl groups. The biological activities of the synthetic compounds are now being tested and several noteworthy informations have been already obtained, which will be reported elsewhere.

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9) This protecting group is resistant against Meerwein's reagent and removable afterwards without affecting other ester functions.
10) Benzylation facilitated the chromatographic purification of the product.
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12) In order to enrich the α-anomer 21, the cleavage product of the corresponding β-allyl glycoside was once dissolved in a mixture of THF-AcOH and recovered by evaporation of the solvent after 24 h.[11]

(Received in Japan 12 February 1983)